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Growth and Sporulation of *Nomuraea rileyi* Isolates on Nitrogen Based Media

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Evaluation of *N. rileyi* isolates on different nitrogen sources media showed potato peptone agar was significantly superior for *N. rileyi* isolates over all other media and supported the maximum biomass, mycelial growth and conidial count. In case of Project Directorate of Biological Control isolate, among the six nitrogen sources tested, the maximum radial growth, biomass and spore production were observed in potato peptone agar medium both in solid as well as in the liquid medium. Similar trend of growth and development was observed with Directorate of Oil seeds Research and local isolates of *N. rileyi.*

Key words: *Nomuraea rileyi*, nitrogen sources, radial growth, biomass, spore production

Biological control of insect pests is one of the most important components of IPM, in which entomopathogens are exploited. Entomo pathogens include a diversity of viruses, bacteria, fungi, protozoans and nematodes that cause disease in insect. Entomopathogenic fungi are more specific and friendly to environment and natural enemies. Entomopathogenic fungi particularly green muscardine fungi *Nomuraea rileyi* (Farlow) Samson is a cosmopolitan species and reported to be pathogenic to several economically important lepidopterous pests. Use of *N. rileyi* is an alternative method to reduce synthetic pesticide use in integrated pest management and sustainable agriculture. Success of any microbial control programmes depends on production of sufficient quantity of inoculum for field application. Efficient production technologies have been developed for laboratory and commercial use of entomopathogenic fungi. Most of the entomopathogens are facultative pathogens and can be mass produced in synthetic, semi synthetic or natural media containing suitable nutrient source. Selection of strains of fungi having high virulence, good growth and sporulation is considered important in mass culturing. Hence the present study was taken up to test six different nitrogen sources based media for their growth and development of *N. rileyi* isolates.

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Materials and Methods

Collection and maintenance of N. rileyi

Pure cultures of *N. rileyi* were obtained from Project Directorate of Biological Control (PDBC), Bangalore and Directorate of Oilseeds Research (DOR), Hyderabad. Surveys were also conducted in cotton, tomato, castor and pulses cropping areas of Coimbatore District (Thondamuthur) and *N. rileyi* infected cadavers were collected and maintained as local isolates. The three isolates of *N. rileyi* (PDBC, DOR and LOCAL) were maintained at the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai. The fungal isolates maintained in the standard mycological media and incubated for 10 days at 25°C, in dishes showing good fungal growth were selected for the experimental inoculation.

Isolation from infected cadavers

Sabouraud's Maltose Agar medium supplemented with 1% Yeast extract (SMAY)

medium was used to isolate the fungus. Conidia of the *N. rileyi* formed on the cadavers were taken by a mycological loop and streaked on SMAY medium. After incubation at room temperature 28 ± 2ºC for a week, the colonies obtained were transferred to SMAY slants for preservation. The isolates were identified by microscopic view and observed for the conidia forming mycelia for conidiogenous structure and conidial morphology (Samson *et al*., 1988; Aoki, 1989). *N. rileyi* isolates were stored at refrigerated condition (4ºC).

Nitrogen source media

The media were prepared from six different nitrogen sources *viz.,* peptone, ammonium nitrate, sodium nitrate, beef extract, urea and thio - urea 20 g / l along with potato extract (200 g /l) and agar (20 g / l). Observations on radial growth, biomass and spore count were recorded as follows.

Radial growth

Growth rate of mycelia in terms of diameter of fungal mat (mm) was assessed on solid medium. A fungal disc measuring 0.5 cm of the respective isolates before sporulation was inoculated at the centre of the plate. Inoculated agar plates were incubated at 25 ± 1ºC for 15 days. Growth of the colony was measured at an interval of 5 days (Hall and Bell, 1961).

Table 1. Influence of nitrogen sources in solid and liquid media on growth and sporulation (PDBC isolate) of *N. rileyi*

Nitrogen source	Solid medium				Liquid medium	
	Radial growth (mm)*					
	5th day	10th day	15th day	Sporulation on15th day (10^7 / ml)*	Biomass (gm)*	Sporulation on 15th day (10^7 / ml)*
Potato peptone agar	6.50[a]	24.00[a]	46.00[a]	2.417[a]	1.32[a]	3.723[a]
				(0.383)	(1.147)	(0.571)
Potato ammonium nitrate agar	6.66[a]	23.33[b]	40.00[c]	1.515[c]	1.03[c]	2.014[c]
				(0.180)	(0.014)	(0.304)
Potato sodium nitrate agar	5.66[b]	22.00[b]	39.50[cd]	1.334[e]	1.01[d]	1.361[e]
				(0.125)	(1.004)	(0.138)
Potato beef extract agar	5.00[c]	20.50[c]	42.50[b]	1.715[b]	1.21[b]	2.102[b]
				(0.234)	(1.100)	(0.323)
Potato urea agar	5.28[b]	19.50[d]	38.50[d]	1.412[d]	0.90[e]	1.9836[d]
				(0.150)	(0.945)	(0.297)
Potato thio urea agar	5.00[c]	15.50[d]	35.00[e]	1.315[f]	0.71[f]	1.315[f]
				(0.119)	(0.839)	(0.119)
CD (P = 0.05)	**0.7956**	**0.7969**	**0.5362**	**0.0014**	**0.0045**	**0.0011**
SEd	**0.3651**	**0.3658**	**0.2461**	**0.0007**	**0.0020**	**0.0005**

Figures in parentheses represent log transformation (spore yield) and square root transformation (biomass).
Means in a column followed by same superscript letters are not significantly different according DMRT at P = 0.05.
* Values are mean of five replications;
PDBC – Project Directorate of Biological control, Bangalore.

Biomass production and spore count

Fifty millilitres of the different nutrient sources medium were transferred to 250 ml conical flasks in 5 replicates without agar. Flasks were plugged with non absorbent cotton and sterilized at 15psi for 20 min. After cooling 10 mm discs of the fungus grown on the respective mycological medium in petri dishes were cored out using a flame sterilized cork borer under aseptic condition and incubated for 15 days. After incubation at 25 ± 1ºC the individual broth cultures were filtered through pre – weighed Whatman No. 1 filter papers. The mycelial mats collected on the filter papers accounted for the dry mycelial weight or biomass (Hall and Bell, 1961).

Fungal mat was macerated with pestle and mortar using 0.02 per cent Tween 20 (Polyethylene sorbitan monolaureate) as an emulsifier to get uniform spore suspension. Spores were further extracted by passing the suspension through a muslin cloth. The filtrate was assessed with the help of improved Neubauer's haemocytometer (Jones, 1962).

Results and Discussion

The radial growth, biomass and spore production of *N. rileyi* isolates varied significantly with various nitrogen source media tested and the results are shown in tables.

PDBC isolate

The potato peptone agar medium recorded maximum radial growth of 46.00 mm followed by potato beef extract agar medium (42.50 mm) on 15th day. The least radial growth was observed in potato thio urea agar medium (35.00 mm). In solid medium, the potato peptone agar medium

Table 2. Influence of nitrogen sources in solid and liquid media on growth and sporulation (DOR isolate) of *N. rileyi*

Nitrogen source	Solid medium				Liquid medium	
	Radial growth (mm)*			Sporulation on15th day (10^7 / ml)*	Biomass (gm)*	Sporulation on 15th day (10^7 / ml)*
	5th day	10th day	15th day			
Potato peptone agar	7.50^{a}	23.08^{a}	43.50^{a}	2.317^{a}	1.54^{a}	3.753^{a}
				(0.364)	(1.242)	(0.574)
Potato ammonium nitrate agar	6.33^{b}	22.50^{a}	35.50^{d}	1.878^{b}	1.20^{b}	2.173^{c}
				(0.274)	(1.096)	(0.337)
Potato sodium nitrate agar	5.63^{cd}	19.57^{c}	39.00^{bc}	1.335^{e}	1.00^{d}	1.513^{f}
				(0.125)	(0.993)	(0.179)
Potato beef extract agar	5.25^{de}	20.50^{b}	41.25^{b}	1.578^{c}	1.10^{c}	2.493^{b}
				(0.198)	(1.050)	(0.396)
Potato urea agar	6.28^{bc}	19.91^{bc}	39.47^{bc}	1.412^{d}	0.74^{e}	1.870^{d}
				(0.149)	(0.857)	(0.272)
Potato thio urea agar	5.10^{e}	16.60^{d}	34.50^{d}	1.328^{f}	0.72^{f}	1.711^{e}
				(0.123)	(0.849)	(0.233)
CD (P = 0.05)	**0.5602**	**0.8792**	**1.1204**	**0.0012**	**0.0004**	**0.0020**
SEd	**0.2571**	**0.4035**	**0.5142**	**0.0005**	**0.0002**	**0.0009**

Figures in parentheses represent log transformation (spore yield) and square root transformation (biomass).
Means in a column followed by same superscript letters are not significantly different according to DMRT at P = 0.05.
* Values are mean of five replications;
DOR – Directorate of Oilseeds Research, Hyderabad.

was significantly superior in spore production which was 2.417 x 10^7 spores per ml. The least sporulation was observed in potato thio urea agar (1.315 x 10^7 spores per ml) in the order of effectiveness (Table 1). In liquid medium, the biomass production was found to be higher in potato peptone agar (1.32 gm). The lowest biomass yield was recorded in potato thio urea agar (0.71 gm). Similar trend was observed in sporulation also. The maximum spore production was observed in potato peptone agar medium (3.723 x 10^7 spores per ml). This was followed by potato beef extract agar (2.102 x 10^7 spores per ml) and then by potato ammonium nitrate agar (2.014 x 10^7 spores per ml). The lowest sporulation was observed in potato thio - urea agar (1.315 x 10^7 spores per ml) (Table 1).

DOR isolate

In solid medium, the maximum radial growth was recorded in potato peptone agar medium (43.50 mm) on 15th day. The minimum radial growth was observed in potato thio - urea agar (34.50 mm). The potato peptone agar medium was significantly superior in spore production which was 2.317 x 10^7 spores per ml followed by potato ammonium nitrate agar (1.878 x 10^7 spores per ml), the lowest spore production was observed in potato thio - urea agar (1.328 x 10^7 spores per ml) (Table 2). In case of liquid medium, the maximum biomass was recorded in potato peptone agar (1.54 gm). The minimum biomass production of 0.74 gm and 0.72 gm were observed in potato urea agar and potato thio - urea agar media. The maximum spore production was observed in potato peptone agar medium (3.753 x 10^7 spores per ml). The minimum spore production was observed in potato sodium nitrate agar (1.513 x 10^7 spores per ml) (Table 2).

Table 3. Influence of nitrogen sources in solid and liquid media on growth and sporulation (Local isolate) of *N. rileyi*

Nitrogen source	Solid medium				Liquid medium	
	Radial growth (mm)*			Sporulation on15th day (10^7 / ml)*	Biomass (gm)*	Sporulation on 15th day (10^7 / ml)*
	5th day	10th day	15th day			
Potato peptone agar	5.66^{a}	17.70^{a}	40.81^{a}	2.537^{a}	1.28^{a}	3.984^{a}
				(0.404)	(1.129)	(0.600)
Potato ammonium nitrate agar	5.16^{c}	16.41^{c}	36.50^{d}	1.675^{d}	1.14^{b}	2.105^{c}
				(0.224)	(1.060)	(0.323)
Potato sodium nitrate agar	5.16^{c}	14.68^{d}	38.75^{c}	1.315^{e}	1.00^{d}	1.787^{e}
				(0.117)	(0.999)	(0.252)
Potato beef extract agar	5.33^{b}	18.83^{b}	40.00^{b}	1.812^{b}	1.12^{c}	2.373^{b}
				(0.258)	(1.059)	(0.375)
Potato urea agar	5.10^{cd}	13.50^{e}	36.00^{e}	1.724^{c}	0.63^{e}	2.025^{d}
				(0.237)	(0.795)	(0.306)
Potato thio urea agar	5.00^{d}	12.00^{f}	35.00^{f}	1.314^{e}	0.60^{f}	1.326^{f}
				(0.117)	(0.774)	(0.122)
CD (P = 0.05)	0.1258	0.4537	0.7263	0.0012	0.0003	0.0008
SEd	0.0577	0.1991	0.3333	0.0005	0.0001	0.0004

Figures in parentheses represent log transformation (spore yield) and square root transformation (biomass).
Means in a column followed by same superscript letters are not significantly different according to DMRT at P = 0.05.
* Values are mean of five replications;
Local isolate collected from Coimbatore (Thondamuthur).

Local isolate

Excellent growth of the fungus was obtained with potato peptone agar (40.81 mm) on 15th day. Potato urea agar and potato thio - urea agar recorded comparatively lesser growth of 36.00 mm and 35.00 mm, respectively. Potato peptone agar was found significantly superior than other media by recording abundant sporulation of 2.537 x 10^7 spores per ml. Potato thio - urea agar produced comparatively lower sporulation (1.314 x 10^7 spores / ml). The maximum biomass was recorded in potato peptone agar medium (1.28 gm) and the minimum biomass production of 0.60 gm was observed in potato thio urea agar media (Table 3). In liquid medium, the spore production was significantly different in various culture media tested. The maximum spore production was observed in potato peptone agar medium (3.984 x 10^7 spores per ml) and the least spore yield was recorded in potato thio - urea agar with 1.326 x 10^7 spores per ml (Table 3).

In the present investigation, potato peptone agar medium recorded the maximum radial growth, biomass and spore production than other nitrogen source media tested. This is also in confirmity with earlier work of Kumar *et al*. (2003) who reported that maximum mycelial growth was obtained in SMAY containing protease peptone or mycological peptone, while, conidiation was observed in SMAY containing these peptone sources. Earlier various carbon and nitrogen sources tested were corn flour, corn starch, rice hull, glycerol and sucrose (Mazumdar *et al*., 1995). The highest wet weight was obtained in medium containing mycological peptone, while the lowest in the one with protease peptone. Mycelia and conidia were observed in liquid and solid media containing bacteriological peptone. Vimala Devi *et al*. (2000) found that the maltose and peptone, carbon and nitrogen sources could be effectively replaced with two per cent barley extract and one per cent soybean extract, was found to be significantly superior in increasing the growth and sporulation. To optimize the growth and sporulation, the carbon, nitrogen, mineral and pH levels may require precise balancing (Gupta and Mukerjii, 2000).

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